Kidney International, Vol. 41 (1992), pp. 741–748

Glomerular structure in type-1 (insulin-dependent) diabetic patients with normo- and microalbuminuria

JAMES D. WALKER, COLLIN F. CLOSE, SHARON L. JONES, MARTIN RAFFTERY, HARRY KEEN, GIANCARLO VIBERTI, and RUTH ØSTERBY

Units for Metabolic and Renal Medicine, UMDS, Guy's Hospital, London, England, United Kingdom; and Electron Microscopy Laboratory, Institute for Experimental Clinical Research, University Institute of Pathology, Kommunehospitalet, Aarhus, Denmark

Glomerular structure in type-1 (insulin-dependent) diabetic patients with normo- and microalbuminuria. Kidney biopsies from 15 type-1 (insulin-dependent) diabetic patients with a range of albumin excretion (AER) were analyzed. Nine patients had normal AER, and six had microalbuminuria. Basement membrane thickness, BMT, and mesangial matrix volume fraction, V_V(mat/glom), were obtained from at least three glomeruli per biopsy. Mesangial structures were estimated with electron microscopic analysis at three levels in each glomerulus. Glomerulopathy parameters were significantly increased in micro- versus normoalbuminuric patients with the following means and (CV): BMT 571 nm (0.12) and 442 nm (0.25), $P = 0.03$; V_V(mes/glom) 0.31 (0.20) and 0.22 (0.14), $P = 0.002$; V_V(matrix/glom) 0.17 (0.25) and 0.11 (0.28), $P = 0.006$; matrix star volume 56 μm^3 (0.47) and 22 μm^3 (0.43), $P = 0.02$. A positive correlation obtained between AER and each of the glomerulopathy parameters, BM thickness, V_V(mes/glom) and V_V(matrix/glom), as well as between AER and a structural index expressing the sum of changes in the peripheral BM and in the mesangium ($r = 0.62$, $P = 0.01$). The results indicated a parallel course of mesangial and peripheral BM changes: a positive correlation obtained between BM thickness and mesangial parameters [BMT versus V_V(matrix/glom): $r = 0.82$, $P = 0.0001$] and the ratio of the two subsets of glomerular BM material (PBM:matrix) did not show significant difference between normo- and microalbuminuric groups. The data give strong support to the contention that the transition from normo- into the microalbuminuric phase is linked to progressing glomerulopathy.

The earliest clinical sign of diabetic nephropathy is an elevation of the urinary albumin excretion rate. A slight increase to above the normal range (microalbuminuria) indicates a high risk of developing nephropathy [1–3]. Overt diabetic nephropathy with proteinuria and decreasing glomerular filtration rate (GFR) is closely linked to advanced diabetic glomerulopathy [4, 5], and it would seem likely that the stage of microalbuminuria is related to an early stage of glomerulopathy. The structural lesions accompanying the transition from normo- to microalbuminuria have not previously been identified. An extensive study of glomerular structure in diabetic patients with and without microalbuminuria failed to find significant differences in the structural parameters between the two subgroups in the absence of a raised blood pressure or reduced creatinine clearance [6].

The structural lesions of diabetic glomerulopathy develop very slowly over years. Incipient nephropathy is therefore preceded by a stage of silent glomerulopathy the length of which is remarkably different between patients. Furthermore, normoalbuminuric diabetic patients include the subset of cases who will never develop nephropathy, constituting about 60 to 70% of the patients [7].

Further structural studies of the preclinical phase, and in particular of the transition phase from normo- to microalbuminuria, therefore seem relevant. For the detection of early glomerulopathy it is necessary to have sensitive measures of glomerular changes. This has previously been a problem because of the estimation of mesangial changes [6, 8, 9]. We have therefore undertaken the present study in type-1 diabetic patients who show a range of albumin excretion. In this series we have taken particular care to improve the estimation of the mesangial regions.

Methods

Patients

Thirty-two patients involved in a study of the natural history of diabetic nephropathy in insulin-dependent diabetes (MCS Study) were asked to participate in the study which was approved by the Ethics Committee of Guy's Hospital. After a full discussion concerning the procedures involved, 18 gave their informed written consent.

The patients were admitted to a metabolic ward on the day prior to the biopsy. Blood pressure was taken in the right arm using a Hawksley random zero sphygmomanometer after 10 minutes of rest.

Two readings to the nearest 2 mm Hg were taken and the mean calculated. GFR was calculated from the clearance of ^{51}Cr EDTA after a single intravenous injection [10] and corrected for a body surface area of 1.73 m^2. The urinary albumin excretion rate was measured from a timed overnight collection and a mid-stream specimen cultured to exclude infection. All patients involved in the study had three overnight urinary albumin excretion rate (ONAER) measurements performed during the year preceding the biopsy. An ONAER below 20 μg/min was taken to indicate normoalbuminuria and a level greater than 20 μg/min to indicate microalbuminuria. In the nine

Table 1. Clinical details of 15 insulin-dependent diabetic patients with normoalbuminuria and microalbuminuria

Case no.	Sex	Age	DDM	BP	HbA_1	AER	GFR
		years		*mm Hg*	%	*μg/min*	*ml/min/1.73 m²*
Normoalbuminuric patients							
1	F	30	5	106/56	7.05	2.1	116
2	F	21	8	109/73	6.05	3.4	170
3	M	34	21	119/74	7.8	3.7	123
4	M	39	31	122/60	7.3	4.5	116
5	M	38	5	127/71	7.5	5.2	158
6	F	22	11	122/51	6.6	5.2	113
7	M	37	12	142/102	8.2	6.9	125
8	F	19	7	126/74	9.7	7.2	176
9	M	33	12	110/77	7.0	7.2	96
	4F, 5M						
Mean		30	12	120/71	7.5	5.0	129
SD		7.5	8.5	11/15	1.0	1.8	24
Microalbuminuric patients							
10	F	21	13	130/76	10.05	22.5	178
11	F	55	36	118/64	7.05	25.0	52
12	M	32	18	131/87	9.0	47.6	127
13	M	52	15	138/88	8.6	65.1	117
14	F	43	22	90/52	9.5	93.5	193
15	M	26	10	118/62	8.2	179.7	142
	3F, 3M						
Mean		38	19	121/72	8.7[a]	72.2	135
SD		14	9	17/14	1.0	58.9	50

[a] $2P = 0.045$ for HbA_1 between normo- and microalbuminuric groups.

patients with normoalbuminuria all recorded ONAER levels were less than 20 μg/min, whereas in the six patients with microalbuminuria no recorded levels during the previous year were less than this threshold. The ONAER measurements quoted are those recorded during the night prior to the renal biopsy. Glycosylated hemoglobin was assayed using the Corning method (Ciba-Corning, Halstead, Denmark).

The clinical details of the patients are given in Table 1. The patients are classified according to their albumin excretion rates. Cases 1 through 9 have albumin excretion rates below 20 μg/min and are classified as normoalbuminuric; cases 10 through 15 have albumin excretion rates >20 and <200 μg/min, and are classified as microalbuminuric. In one case the biopsy was poorly fixed and structural measurements were not technically possible. Two patients were frankly proteinuric and were excluded from the analysis.

The patients in the normoalbuminuric group were slightly younger and had a shorter duration of diabetes than the microalbuminuric group, although these differences were not statistically significant. Blood pressure was similar between the two groups, however, the remarkably low level in one microalbuminuric patient tended to skew this group. Glycemic control, as assessed by HbA_1, was better in the normoalbuminuric group, and by definition urinary albumin excretion rates were appreciably higher in the microalbuminuric subjects.

Biopsies

A percutaneous renal biopsy was taken using a Tru-cut needle under ultrasound guidance. The procedure was uncomplicated except for one case in which frank hematuria followed the procedure, but settled spontaneously within 12 hours.

The entire cylinder was immediately immersed into the fixative, 2% glutaraldehyde in modified Tyrode buffer [11], and was mailed in the fixative to the EM laboratory in Aarhus. Cutting the tissue into small blocks, dehydration and embedding in Vestopal were carried out after a fixation time of four to seven days (mean 5.9), one to two days in the mail, and thereafter storage at 4°C.

Obtaining structural quantities

Structural quantities relevant to diabetic glomerulopathy were estimated. The structures in question are the peripheral basement membrane (PBM), the mesangial regions and the mesangial matrix. With the primary aim of improving the precision in the estimate of mesangial volume fraction the following procedure was derived:

Sampling from the tissue blocks. Sections of 1 μm thickness were cut, picked up and stained with toluidine blue for light microscopy. The first section in the block, the baseline section, was not used for sampling. Sequential sections with 10 μm intervals were inspected, and any new glomerulus appearing in one of these sections was sampled for analysis. Thin sections were cut at three sequential levels, 60 μm and multiples thereof, from the baseline section. Using this procedure the glomeruli were sampled independently of size or structure, and ultrastructure was studied in the set of three parallel cross sections with a random position along the glomerular diameter. The sections stained for light microscopy (10 μm intervals) were used for the determination of glomerular volume (unpublished data).

This protocol was only introduced in the latter part of the study. The first 11 biopsies were initially studied by sampling one level in each of three glomeruli, using a protocol that ensured an unbiased sampling of glomeruli as well as the level within the glomeruli [12]. The data from these three random levels were subsequently supplemented with data from another two glomeruli, each studied at three levels. Completion of the

Table 2. Material for V_V (mes/glom) and surface estimation

Case no.	No. of glomeruli studied at 1 level	2 levels	3 levels	Total no. of glomeruli represented
1	3		2	5
2	3		2	5
3			3	3
4	3		2	5
5	1	1	3	5
6	3	1	2	6
7			4	4
8	3		2	5
9	3		2	5
10	3		2	5
11	1	1	1	3
12	3		2	5
13	3		2	5
14	4			4
15	3		2	5

precept was not possible in all cases; the number of glomeruli and of levels within glomeruli that were actually obtained in each biopsy are shown in Table 2.

Quantitation by electron microscopy

The glomerular profiles were recorded with overlapping micrographs to produce photomontages at a final magnification of 2350×.

Mesangial volume fraction. This was estimated by point counting, using an 8:1 grid, counting hits on mesangium with the fine points, and hits on the reference space, defined as the circumscribed, minimal convex polygon [5] with the coarse points. The term 'glomerulus' is used in the following for the reference space thus defined. Further, coarse points hitting the glomerular tuft, defined as all structures circumscribed by the base of the epithelial foot processes, were counted. The tuft is the reference space common to low and higher magnification electron microscopy. The distance between coarse points in the grid corresponded to 22 μm.

Surface of mesangial regions. This was estimated by counting intersections between test lines and the mesangium/urinary space interface. The surface density of the boundary equals: 2 × intersections per test line length within the reference space, in this case the glomerular tuft. The surface density estimate was used for the calculation of the derived quantity, matrix T (see below).

From the largest of the three profiles of the level-sectioning, and from each of the three single profiles in each of three glomeruli, a set of micrographs was obtained at a higher magnification (9,990×). The area covered corresponded to about 50% of the total profile and was obtained by systematic random sampling. The micrographs were taken at fixed settings of the specimen stage movement controls. This set of micrographs was obtained in three glomerular profiles in all cases, except case 14 in whom four glomeruli were sampled, and in case 13 a total of five glomeruli were sampled. The set was used for the following estimates.

Thickness of the peripheral basement membrane (BM). The delineation between peripheral BM and the mesangial matrix had a geometrical definition: the dividing line was the perpendicular to the epithelial side of the BM, separating the simple three-layered peripheral BM (endothelium, BM, epithelium) from the more complex mesangial matrix. Measuring points were sampled independently of the BM appearance with a line grid, measurements taken at all places of intersection between grid lines and the endothelial-BM interface. Orthogonal intercepts, that is, the perpendicular distance from endothelial to epithelial cell membrane, were classified at these points [13]. Further, 'true BM thickness' was estimated [14] at the sampling places, where the distinctness of the epithelial cell membrane showed that the section was perpendicular to the BM surface. In this paper the true BM thickness is applied.

Matrix volume fraction. For the purpose of quantitation, mesangial matrix was defined as all extracellular material in the mesangial regions. An integral test system was used to count points hitting matrix, mesangial regions, and the glomerular tuft (definition as at low magnification). The grid used for this counting had a 4:2:1 set of points.

Matrix star volume. This estimate was obtained by measuring linear intercepts in the matrix, from random points and with a uniform 3-D orientation distribution [15] (Fig. 1). In practice, the intercepts were measured on the micrographs, sampling the measuring points with the point-grid, and measuring in a predetermined direction on the micrograph. The uniform 3-D orientation of intercepts within the matrix was obtained in the sectioning, and in sampling of micrographs. The point-sampled intercepts (ℓ_o) were classified with a ruler that has broad lowest class and narrow higher classes. All calculations were carried out in the cubed-scale (ℓ^3). The matrix star volume is:

$$V^* = \frac{\pi}{3} \cdot \overline{(\ell_o^3)}$$

Derived structural quantities

Matrix-T, ("thickness"). An estimate of the (imaginary) matrix thickness (Fig. 2) was obtained from V_V(matrix/tuft)/S_V([mat/urinary space]/tuft). The dimension of the estimate is $1/\mu^{-1} = \mu$, and it corresponds to the thickness of the matrix, if it were spread out in an even layer on the mesangial/urinary space surface. That is, as the star volume it is an absolute and not a relative measure, which is in contradistinction to the volume fraction.

Structural index. To express the sum of structural changes of diabetic glomerulopathy the following index was calculated:

$$\text{Index(structure)} = \text{BMT}/10 + V_V(\text{matrix/glom}) \cdot 100$$

Statistics

Comparisons between the two subsets of patients, normo- and microalbuminuric patients, respectively, were done by two-tailed *t*-tests, and correlations by least squares regression (BMDP, SOLO statistical System, version 2.0).

Results

Structural quantities in the individual biopsies are presented in Table 3. Comparison of the two subgroups, with normal albumin excretion (cases 1 to 9) and with microalbuminuria (cases 10 to 15), respectively, showed statistically significant

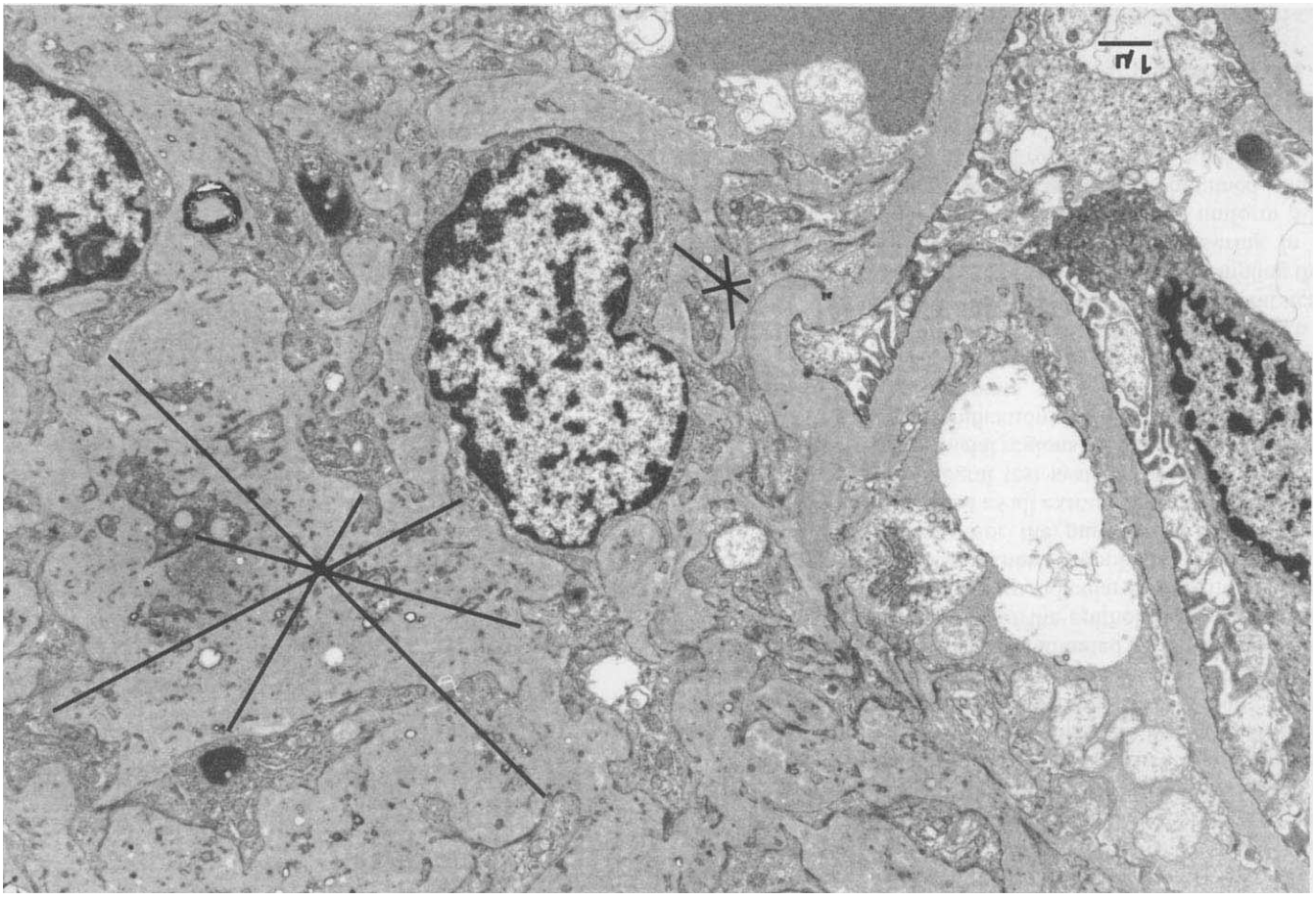

Fig. 1. *Glomerular segment from case no. 14.* The matrix appears on section as isolated, irregular islands. The star volume can be imagined 3-dimensionally as the average volume that can be seen from random points within the matrix. The intercepts that are drawn within two matrix segments illustrate this on the 2-D section.

differences in each of the parameters: thickness of the peripheral basement membrane, mesangial and mesangial-matrix volume fractions, matrix star volume and matrix "thickness."

The coefficient of variation, CV (= SD/mean), of BM thickness was remarkably high in the normoalbuminuric group (0.25). Among the nine cases were some with completely normal, and others with definitely, although moderately, increased values. The coefficient of variation between the three to five profiles within the individual biopsies is also shown in Table 3.

Basement membrane thickness was estimated with the orthogonal intercept method as well as with direct measurements of 'true thickness' in 10 cases. The coefficient of correlation between the two was 0.86. Considering the two estimates as 'double determinations' the coefficient of error was 0.02.

The distribution of extracellular material between the two subsets, peripheral BM and mesangial matrix V_V(PBM/[PBM + matrix]), was not significantly different between the two groups (Table 3), although there was a tendency to matrix dominance in the microalbuminuric patients.

Each of the structural parameters showed a positive correlation with the albumin excretion rate. Figure 3 shows the relationship between the structural index as the independent variable, and AER. The coefficient of correlation is 0.62 (r^2 = 0.38; P = 0.01).

Thickness of the peripheral BM showed a positive correlation with each of the mesangial quantities. Figure 4 illustrates the relationship to the matrix volume fraction, for which r = 0.82 (r^2 = 0.66; P = 0.0001).

Neither in the normoalbuminuric nor in the microalbuminuric groups was there any correlation between duration of diabetes or systemic blood pressure and the structural quantities. The four patients with mesangial volume fraction above 0.30 had particularly long duration of diabetes, and they were among the oldest in the series.

A few remarkable observations were the following:

Classical Kimmelstiel Wilson nodules were observed in cases no. 12 and 13. Case no. 13 had capillary formation in the capsule of Bowman [16].

In one case (no. 11) glomerular occlusion was prominent. A total of 16 corpuscles were encountered in the blocks, and of these nine were totally sclerosed.

Capsular drops, sometimes very large, were observed in the following cases: 1, 4, 6, 10, 11, 12, 15.

At the ultrastructural level case no. 13 was remarkable in that

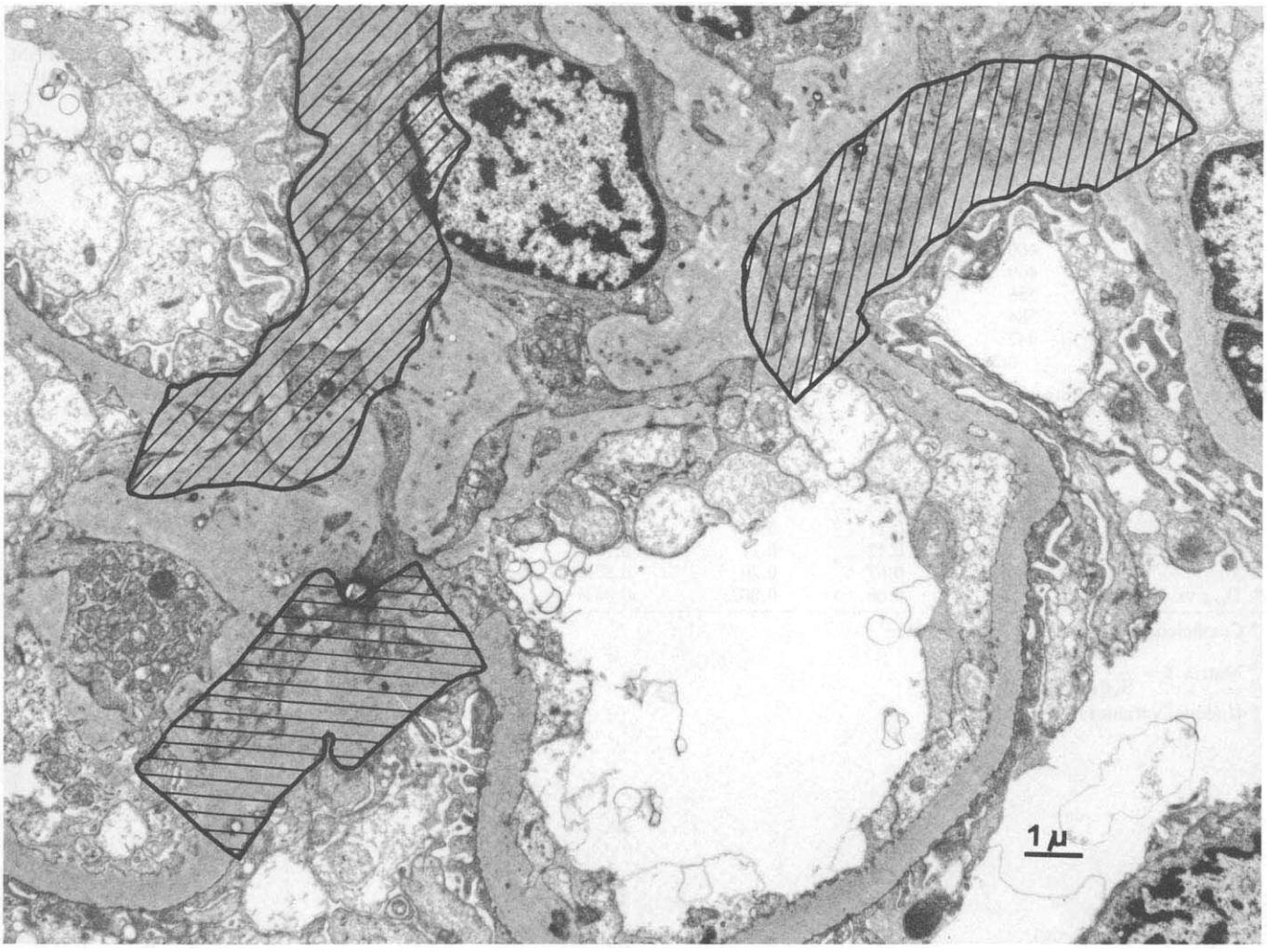

Fig. 2. *The imaginary matrix "thickness" is the height of the layer of matrix if it were evenly spread out on the mesangial-urinary space interface, indicated as the hatched area.*

the peripheral BM showed a highly varying thickness, and its structure was dominated by many fluffy areas [17]. In the mesangial matrix electron-dense areas were present.

Discussion

The data obtained in the present series demonstrated a clear difference between patients with normo- versus microalbuminuria: the latter group had more advanced diabetic glomerulopathy than the former. These results therefore differ from those published for a somewhat larger series [6]. The failure to find a difference in the previous paper may be related to the composition of the patients groups. All patients studied, including those with normoalbuminuria, were candidates for pancreas transplantation. Further, the published results indicate a tendency for BM to be thicker, and as for the estimates of mesangial regions, a very large variation obtained, probably reflecting a low precision in the estimate.

The present results do not provide an explanation for the increased leakiness of the filtration barrier in the microalbuminuric patients. The correlation within this fairly low level of albuminuria between AER and the structural parameters, however, support the contention that the leakiness is a consequence of the structural modifications. It seems very unlikely *a priori* that the current BM thickness or the mesangial volume fraction should be the direct cause of albuminuria, and so a complete accordance between the estimations of glomerulopathy and the clinical state was not to be expected. For this reason we decided to illustrate the relationship between overall glomerular pathology (structural index) and level of AER, advocating the idea that a possible mechanism is modification of the filtration barrier, perhaps in localized areas, which develops as a consequence of the changing glomerular composition. We have been struck by the appearance in many of these glomeruli of isolated, very thin segments of the peripheral BM contrasting markedly to the remainder of the basement membranes. This change in the distribution of peripheral BM thickness needs to be documented by more extensive studies of the BM distributions. It is probably noteworthy that very thin segments were quite prominent in case no. 15, who had a moderately expressed glomerulopathy and yet the highest albumin excretion rate. The thin segments, often close to a mesangial region in the plane of

Table 3. Structural parameters in 15 insulin-dependent diabetic patients with normoalbuminuria and microalbuminuria

Case no	BMT mean, nm	BMT CV[a]	V_V Mes/glom	V_V Mat/glom	Matrix T[b] μm	Matrix star vol. μm^3	V_V [PBM/(PBM + matrix)]
Normoalbuminuric patients							
1	320	0.03	0.20	0.09	1.6	17	0.42
2	323	0.004	0.21	0.08	1.3	19	0.43
3	362	0.04	0.18	0.09	2.9	17	0.36
4	542	0.08	0.25	0.13	2.7	20	0.40
5	334	0.12	0.17	0.07	2.4	19	0.42
6	460	0.07	0.21	0.13	1.7	45	0.44
7	469	0.07	0.22	0.10	1.4	18	0.44
8	586	0.04	0.25	0.13	2.9	27	0.38
9	584	0.07	0.25	0.14	3.1	14	0.46
Mean	442	0.06	0.22	0.11	2.2	22.1	0.42
CV	0.25	0.58	0.14	0.24	0.32	0.43	0.08
Microalbuminuric patients							
10	510	0.07	0.23	0.14	2.8	43	0.42
11	654	0.05	0.37	0.19	4.3	48	0.31
12	643	0.12	0.33	0.20	5.1	57	0.26
13	588	0.05	0.37	0.21	5.5	99	0.22
14	522	0.26	0.32	0.19	4.7	68	0.23
15	506	0.11	0.24	0.10	2.2	20	0.52
Mean	571	0.12	0.31	0.17	4.1	56.3	0.33
CV	0.12	0.67	0.20	0.25	0.32	0.47	0.37
$2P$, D_{NA} vs. D_{MI}	0.03	0.06	0.002	0.003	0.003	0.02[c]	0.12[c]

[a] Coefficient of variation of 3 to 4 profiles

[b] Matrix $T = \frac{V_V(\text{matrix/tuft})}{S_V([\text{mat/us}]/\text{tuft})\mu^{-1}}$

[c] Unequal variances

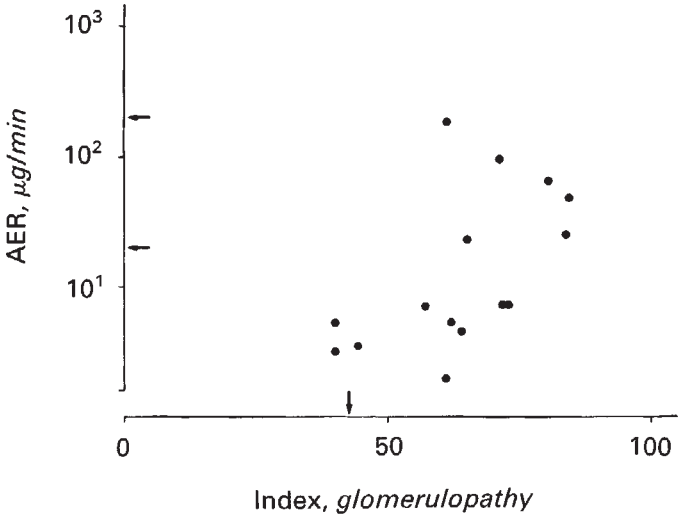

Fig. 3. *Relationship between the index expressing the sum of peripheral basement membrane and mesangial changes: BMT/10 + V_V(matrix/glom) · 100, and albumin excretion rate.* Arrows on the y axis indicate the range of microalbuminuria. Arrow on the x axis shows the mean index as estimated in a group of 8 kidney donors [17]. The coefficient of correlation is $r = 0.62$; $P = 0.01$.

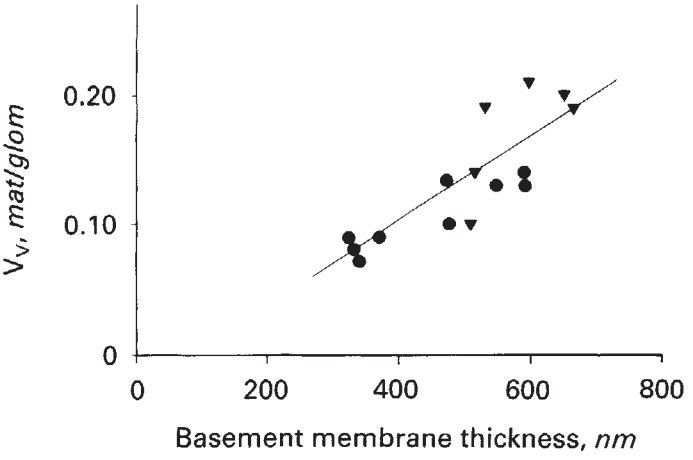

Fig. 4. *The relationship between peripheral basement membrane thickness and mesangial matrix as fraction of glomerulus.* $r = 0.82$, $P = 0.0001$. Symbols are: (●) D_{NA}; (▼) D_{MI}.

sectioning, give rise to association with "new vessel formation", previously observed in more advanced stages of glomerulopathy [16]. If at this stage the glomeruli produce a "second growth spurt," triggered by the progressing glomerulopathy, new vessel formation may be a site of leakage. These vessels, and perhaps other segments of the filtration barrier as well, may be the site of qualitative changes of the basement membrane material [17]. Particular interest has been paid to loss of negatively charged sites [18]. At present, however, the exact mechanism underlying the albuminuria has not been unravelled.

Another point to bear in mind, considering structure-function relationships, is that the structural parameters are estimators, obtained in a renal biopsy. A high consistency has previously been shown comparing the parameters in pair-biopsies from kidney donors [19], but it is unknown to which extent this holds in early stages of diabetic glomerulopathy.

Previous studies have shown that the initially detectable lesion of diabetic glomerulopathy is thickening of the peripheral BM [14], and the clinically very important mesangial enlargement [20] is demonstrated only fairly late in the course. One possibility is that the two lesions are separate, unrelated entities. Alternatively, the common denominator in the diabetic glomerulopathy may be an abnormality in BM metabolism leading to imbalance in synthesis/breakdown ratio, manifested in mesangial matrix and peripheral BM in a parallel course. The present results lend support to this latter contention.

Thickness of the peripheral BM was estimated in only three profiles per biopsy. The very low variation in this estimator which has repeatedly been observed justifies this. It was confirmed in the present series. Applying the 'true-thickness measurement' the thickness can be evaluated in each individual profile and in individual loops, whereas with the orthogonal intercept method [13] the requirement of uniform orientation distribution necessitates results from more (three) glomeruli to be summed for the calculation. The variation between the three individual glomeruli per biopsy in the present series, representing early stages of glomerulopathy and 'normal' glomeruli (in some of the normoalbuminuric patients) was very low in most cases.

Two new measures were included in this study: the matrix star volume and "thickness." No comparisons can therefore be made with other data. As for the matrix thickness it was chosen to 'spread out' the matrix on the mesangial-urinary space interface, thus imagining the glomerular basement membrane material as a continuous lining of the glomerular tuft (Fig. 2). This estimate may be particularly sensitive for the detection of early matrix changes, since it increases with increase in the volume of individual mesangial regions, even assuming unchanged mesangial shape and composition [V_V(matrix/mes)], and it will increase with increasing matrix volume fraction. In the two situations the denominator and the nominator, respectively, increase. In early diabetic glomerulopathy both types of mesangial changes may well occur in concert. The star volume will increase with increasing confluence and/or convexity of the matrix. Such changes are tantamount to increasing separation of the mesangial cells, which may entail fundamental interference with the functions of mesangial regions. The star volume may therefore be an appropriate parameter to follow the development of changes in the progressing diabetic glomerulopathy.

The higher HbA_1 level in the patients with microalbuminuria conforms with findings in larger series of patients [21, 22]. If this reflects a long-standing poorer metabolic control over all the years of diabetes, it may have played a role in the development of structural and functional abnormalities.

The functional data were remarkable in case no. 11, who had only a slightly increased AER, but a low level of GFR. The histological findings in the biopsy of a high fraction of occluded glomeruli—the cause of which is not immediately clear—, tubular atrophy and interstitial fibrosis, may explain the clinical state.

It is important to stress that the group of normoalbuminuric diabetic patients is a heterogeneous group. Some of the cases may eventually develop nephropathy, and therefore probably are developing the early glomerulopathy; others will escape this complication. Whether the present data illuminate prognostically valid risks of this development remains to be evaluated.

Acknowledgments

J.D. Walker and C.F. Close were supported with a fellowship from Juvenile Diabetes Foundation International. The project has further been supported by grants from: Juvenile Diabetes Foundation Int., grant #190592, Helen and Einar Bjørnovs Foundation, The Danish Diabetes Association, The Danish Medical Research Council, Lægevidenskabens Fremme, Bernhard and Marie Klein's Legat, Nordic Insulin Foundation, NOVO Foundation, and Aarhus University Research Foundation. The technical assistance of Ms. K. Gerlach and Ms. B. Saugbjerg is gratefully acknowledged.

Reprint requests to Ruth Østerby, M.D., Electron Microscopy Laboratory, Institute of Pathology, Kommunehospitalet, DK-8000 Århus C, Denmark.

References

1. Parving H-H, Oxenbøll B, Svendsen PAA, Christiansen JS, Andersen AR: Early detection of patients at risk of developing diabetic nephropathy: A longitudinal study of urinary albumin excretion. *Acta Endocrinol* (Copenh) 100:550–555, 1982
2. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H: Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* I:1430–1432, 1982
3. Mogensen CE, Christensen CK: Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 311:89–93, 1984
4. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
5. Østerby R, Parving H-H, Nyberg G, Hommel E, Jørgensen HE, Løkkegaard H, Svalander C: A strong correlation between glomerular filtration rate and filtration surface in diabetic nephropathy. *Diabetologia* 31:265–270, 1988
6. Chavers BM, Bilous RW, Ellis EN, Steffes MW, Mauer SM: Urinary albumin excretion as a predictor of renal structure in type 1 diabetic patients without overt proteinuria. *N Engl J Med* 320:966–970, 1989
7. Borch-Johnson K: Incidence of nephropathy in insulin-dependent diabetes as related to mortality, in *The Kidney and Hypertension in Diabetes Mellitus*, edited by Mogensen CE, Boston, Martinus Nijhoff Publishing, 1988, pp. 33–40
8. Østerby R, Bangstad HJ, Hanssen KF, Mauer SM, Steffes MW, Viberti GC, Walker J: Stereological studies of early phases of diabetic glomerulopathy. (abstract) *Diabetologia* 33:A147, 1990
9. Steffes MW, Barbosa J, Basgen JM, Sutherland DER, Najarian JS, Mauer SM: Quantitative glomerular morphology of the normal human kidney. *Lab Invest* 49:82–86, 1983
10. Chantler C, Garnett ES, Parsons V, Veall N: Glomerular filtration rate measurement in man by the single shot method using ^{51}Cr EDTA. *Clin Sci* 37:811–813, 1969
11. Maunsbach AB: The influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. II. Effects of varying osmolality, ionic strength, buffer system and fixative concentration of glutaraldehyde solutions. *J Ultrastruct Res* 15:283–309, 1966
12. Østerby R, Gundersen HJG: Sampling problems in the kidney, in *Lecture Notes in Biomathematics* (vol. 23), edited by Miles RE, Serra J, Heidelberg, Springer-Verlag, 1978, pp. 185–191
13. Jensen EB, Gundersen HJG, Østerby R: Determination of membrane thickness distribution from orthogonal intercepts. *J Microsc* 115:19–33, 1979
14. Østerby R: Early phases in the development of diabetic glomerulopathy. A quantitative electron microscopic study. (Thesis) *Acta Med Scand* (suppl)574, 1975
15. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sø-

RENSEN FB, VESTERBY A, WEST MJ: Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* 96:379–394, 1988
16. ØSTERBY R, NYBERG G: New vessel formation in the renal corpuscles in advanced diabetic glomerulopathy. *J Diabetic Compl* 1:122–127, 1987
17. ØSTERBY R, PARVING H-H, HOMMEL E, JØRGENSEN HE, LØKKEGAARD H: Glomerular structure and function in diabetic nephropathy. Early to advanced stages. *Diabetes* 39:1057–1063, 1990
18. DECKERT T, FELDT-RASMUSSEN B, BORCH-JOHNSEN K, JENSEN T, KOFOED-ENEVOLDSEN A: Albumin reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 32:219–226, 1989
19. ØSTERBY R, NYBERG G, ANDERSSON G, FRISK B: Glomerular structural quantities in baseline biopsies from cadaveric donor kidney pairs. *APMIS* (suppl)4:130–136, 1988
20. STEFFES MW, ØSTERBY R, CHAVERS B, MAUER SM: Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes* 38:1077–1081, 1989
21. WISEMAN M, VIBERTI G, MACKINTOSH D, JARRETT RJ, KEEN H: Glycaemia, arterial pressure and micro-albuminuria in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 26:401–405, 1984
22. MOGENSEN CE: Hyperfiltration, hypertension, and diabetic nephropathy in IDDM patients. *Diab Nutr Metab* 2:227–244, 1989